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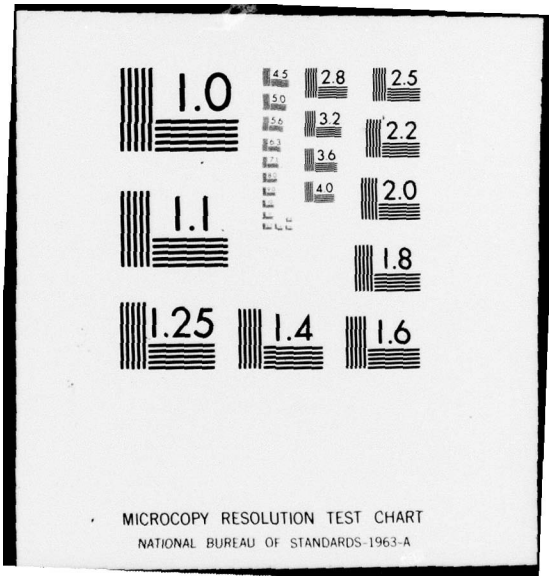
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STUDIES IN THE RAT AND MONKEY ON ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION AND PHARMACOKINETICS OF WR-180,409·H_3PO_4

Final Report

April 1, 1977

Supported by
U. S. Army Research and Development Command
Washington, D. C. 20314

Contract No. DAMD 17-75-C-5065

Principal Investigator - R. L. Furner

Kettering-Meyer Laboratory
Southern Research Institute
Birmingham, Alabama 35205

(Project 3526-VII)

DDC AVAILABILITY

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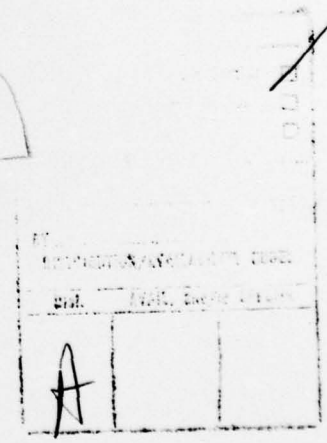

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Final Report for

$WR\text{-}180,409 \cdot H_3PO_4$

STUDIES IN THE RAT AND MONKEY ON ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION, AND PHARMACOKINETICS

The search for new, more-effective antimalarial compounds is a continuing process due to the development of strains of malaria resistant to current therapy. As a part of an integrated effort to obtain information on the absorption, distribution, metabolism, excretion, and pharmacokinetics of investigational new drugs prior to clinical trial, we undertook studies on $WR\text{-}180,409 \cdot H_3PO_4$ [Threo-α-(2-piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridine methanol phosphate].

The studies were carried out using ^{14}C-labeled drug, synthesized by W. H. Yanko of Monsanto Research Corporation and supplied by Dr. R. E. Strube, Department of Organic Chemistry, Walter Reed Army Institute of Research.

A technical protocol was developed in cooperation with the contract monitor in which the experimental design and methodology were described in detail. Two species were studied essentially as shown below.

Long-Evans Rats		Rhesus Monkeys	
Pilot Study	Tissue Distribution Study	Pilot Study (I and II)	Tissue Distribution Study

The objective of the pilot study was to determine the route and rate of drug elimination. Urine and feces from both species were analyzed for $WR\text{-}180,409\text{-}^{14}C \cdot H_3PO_4$ and metabolites. In addition, expired air was collected from rats to determine whether or not the drug might be metabolized to yield volatile products.

In the tissue distribution study we analyzed various organs, tissues, and body fluids for evidence of drug deposition. Products of degradation were quantitatively isolated via methanolic extraction and separated one from another by thin-layer chromatography. Radioactivity was quantified by using liquid scintillation counting techniques.

Rat

Male Long-Evans rats weighing 161-182 g were housed individually in stainless-steel metabolism cages or in Delmar-Roth metabolism cages after oral dosing (20 mg/kg) with an aqueous solution

of WR-180,409-$^{14}C \cdot H_3PO_4$. Urine and feces were collected every 24 hours for all animals and the expired air was monitored for those animals maintained in Delmar-Roth metabolism cages. At 12 days after dosing, the excretion of drug-equivalents in the feces was less than 1% of the dose administered per day. Total excretion of drug-equivalents in the urine was less than 4% of the dose. By day 6 after dosing, excretion in the urine was approximately 0.1% of the dose per day. Excretion via the respiratory route was not significant—only 0.17% of the total dose administered was recovered in 5 days. The recovery of drug-equivalents in the urine and feces over a 51-day period amounted to approximately 65%. Only 1% of the dose remained in the carcass at 51 days after dosing.

After determining the rate and route of excretion of WR-180,409-$^{14}C \cdot H_3PO_4$ and its metabolites, it was desirable to examine the disposition of the compound within the body. Male Long-Evans rats were dosed orally with WR-180,409-$^{14}C \cdot H_3PO_4$ and sacrificed at 3-day intervals through 18 days. The tissues were excised and extracted with methanol for chromatography or assayed by oxidation techniques to determine the total drug-equivalents present. The concentration of WR-180,409-$^{14}C \cdot H_3PO_4$ equivalents was lower in whole blood, plasma, and packed red cells than in other tissues. The highest concentrations of drug-equivalents (after 3 days) were found in lungs (163 µg-eq/g), liver (35 µg-eq/g), spleen (33 µg-eq/g), adrenal gland (5 µg-eq/g) and eyes (13 µg-eq/g). The nature of drug deposition in the lungs is unknown, but the concentration of drug-equivalents in the lung was 94 times the concentration of drug-equivalents in whole blood at 3 days after dosing. Even after 18 days, the concentration of drug-equivalents in the lung was 40 times higher than in whole blood. In the rat, the drug-equivalents were concentrated in glandular tissue such as the submaxillary salivary gland, the adrenal gland, and the lacrimal gland.

The concentration of drug-equivalents was higher in whole blood or plasma than in erythrocytes. The biological half-life ($t_{\frac{1}{2}}$) of elimination of drug-equivalents from erythrocytes was 5.7 days; from plasma, 3.9 days; and from whole blood, 4.0 days. Clearance from the bone marrow was faster ($t_{\frac{1}{2}}$ = 2.5 days) and from the eyes slower ($t_{\frac{1}{2}}$ = 6.2 days) than from blood. Disappearance from the liver ($t_{\frac{1}{2}}$ = 4.5 days) was at a rate similar to the disappearance from plasma and whole blood. Elimination from the tissues examined (days 3-18) was by first-order kinetics; earlier phases (if present) were not detectable in these experiments.

In vivo degradation of WR-180,409-$^{14}C \cdot H_3PO_4$ was assessed through the chromatographic analysis of rat urine, fecal, or tissue extracts.

Using a chromatography system that was developed after the formulation of the technical protocol, we detected the presence of at least 5 metabolites in urinary, fecal, and tissue extracts.

Parent compound represented 8-12% of the total drug-equivalents excreted in the urine, and 35% of the drug-equivalents present in feces. In tissue extracts, the greatest percentage of parent compound (of the total drug-equivalents present) was found in extracts from the lung and in the contents of small intestine (79 and 51% respectively). Smallest percentages of parent compound were found in the contents of cecum and large intestine. Extracts of the contents of the cecum and large intestine contained a major component at R_f .10 which represented 39 and 24%, respectively, of the total drug-equivalents present in those extracts, while liver and contents of small intestine contained this material (R_f .10) in amounts of 5 and 8%, respectively. This presented the question as to whether or not further metabolism occurred in the contents of the cecum and large intestine. Data obtained in the studies with monkeys suggested that further degradation did occur in the gut.

Monkey

Male and female rhesus monkeys assigned for use in these studies were housed in the primate colony at Southern Research Institute. In the first part of the pilot study, 4 female monkeys were dosed orally with 20 mg/kg WR-180,409-$^{14}C \cdot H_3PO_4$. Blood, urine, and feces were collected daily, assessed for total radioactivity, and prepared as needed for analysis by thin-layer chromatography. In the second part of the pilot study, a partial crossover experiment was carried out whereby two monkeys were dosed orally and two were dosed intravenously. In the tissue distribution study, all 4 male monkeys were dosed orally.

Urinary excretion accounted for 20-30% of the dose of WR-180,409-$^{14}C \cdot H_3PO_4$ administered. Fourteen days after dosing, the rate of urinary excretion was less than 0.1%/day of the dose administered. The maximum rate of urinary excretion from monkeys occurred between 12 and 24 hours in pilot study-part I and between 24 and 48 hours in pilot study-part II. There was no difference in the rate of urinary excretion as a result of redosing or between oral and intravenous dosing. Drug-equivalents excreted in the urine were primarily metabolites and so the rate of excretion was not a function of total drug-equivalents present in the animal, but rather, was a function of the rate at which those equivalents were metabolized to the proper form. The rate of elimination of drug equivalents in the urine declined as a first order process, and was independent of the route of drug administration.

Excretion of drug-equivalents in feces from monkeys accounted for 52-59% of the dose of WR-180,409-$^{14}C \cdot H_3PO_4$ administered. Fecal excretion at 14 days after dosing was generally less than 0.2%/day of the dose given. Maximum fecal excretion of drug-equivalents occurred after 48-72 hours. There was no marked change in the rate of excretion of drug-equivalents in feces as a result of redosing. The rate of fecal excretion declined as first-order process.

The rate of elimination of parent compound in urine declined in a biphasic fashion after oral or i.v. dosing. The terminal phase rate constant (Table 13) was higher after i.v. dosing (1.37 day^{-1}) than after oral dosing (0.58 day^{-1}). Estimated total excretion of parent compound was greater after i.v. dosing (977 µg) than after oral dosing (737 µg).

The rate of fecal elimination declined in a monophase with a decay constant of 0.59 day^{-1}. Estimated total excretion was 10,500 µg or approximately 13% of the dose administered.

Although urinary excretion accounted for 20-30% of the total drug-equivalents eliminated, parent compound never accounted for more than 9% of those drug-equivalents. Six other products were consistently present in urine in addition to parent compound with the major product (83% between 12 and 24 hours) remaining at the origin in the solvent system used. After redosing, the chromatographic picture was essentially unchanged.

In fecal extracts, there were also six materials found in addition to parent compound. At 24 hours after dosing, parent compound represented 51% of the total drug-equivalents excreted in feces. However, the excretion of parent compound represented a declining percentage of total drug-equivalents excreted at later time periods. At early time periods, material at the origin comprised about 25-30% of the total in fecal extracts while in urinary extracts 70-85% of the total drug-equivalents remained at the origin.

Approximately 80% of the dose given was accounted for in methanolic extracts of urine and feces. However, 5-10% of the dose remained in fecal solids and was not extractable with 200-300 volumes of methanol. The presence of a material poorly soluble in methanol helps to account for the low recovery of drug-equivalents (<90%) in this and in previous studies. One hour after oral administration of WR-180,409-$^{14}C \cdot H_3PO_4$, low concentrations of drug equivalents were detected in whole blood, plasma, and red blood cells. Although some variation in absorption was observed, peak levels occurred in all orally dosed animals at 8-12 hours after drug administration. High levels persisted through 48 hours and then declined monophasically. Half-lives in plasma, red blood cells and whole blood were approximately 5, 4 and 4 days respectively.

Following i.v. dosing there were three phases of elimination of drug equivalents derived from WR-180,409-$^{14}C \cdot H_3PO_4$. Half-lives for plasma were 0.003, 0.16 and 6.17 days for the α, β, and γ phases respectively. Half-lives for whole blood were similar to those for plasma. Elimination from the red cells was slower.

The maximum concentration of drug equivalents in whole blood, plasma or RBC never exceeded 2.5 µg-equivalents/g as determined by sample combustion analysis.

Tissues, contents of tissues, and fluids were examined in each of 4 monkeys at 1, 3, 6, and 9 days after drug administration. Twenty-four hours after dosing, the highest concentration of drug-equivalents was in bile (541 µg/g) and the lowest in spinal fluid (0.01 µg/g). Vitreous humor (0.25 µg/g) contained fewer drug-equivalents than did whole blood (2.09 µg/g), aqueous humor (2.17 µg/g), or plasma (2.21 µg/g). All other tissues examined contained substantially higher concentrations of drug-equivalents than did whole blood. Affinity for fat, skin, and skeletal muscle was lower than for most other tissues, but was still 4 to 6 times higher than the concentrations found in blood. Considerable sequestration of drug-equivalents was evident after 9 days in lung, liver, pancreas, adrenals, spleen, sciatic and brachial nerves, lumbar spinal cord, and eye. Since neither aqueous nor vitreous humor contained significant concentrations of drug-equivalents, it was concluded that the drug-equivalents were associated with the cellular fraction. Disappearance of total drug-equivalents from the various tissues, tissue-contents, and fluids did not appear to follow first-order kinetics. However, the small number of data points available and the biological variation inherent in the rhesus monkey population precluded a definitive pharmacokinetic analysis.

Parent compound comprised 45% of the total drug-equivalents in whole blood at 24 hours after drug administration. The percentage declined with time to 28% after 9 days. There were 6 products of degradation present in varying amounts in each of the various tissue and/or fluid extracts analyzed.

For example, the major chromatographic component of bile was found at the origin (97% at 24 hours), but a material traveling near the solvent front (R_f .90) comprised 15% of the total drug-equivalents excreted in bile 6 days after drug administration. Bile contained less than 5% of the drug-equivalents as parent compound, in sharp contrast to the liver, which contained 32-67% of the drug equivalents as parent compound. This may point to selective biliary excretion of a particular metabolite(s) by the liver. A similar situation was noted for the kidney which contained high percentages of parent compound while extracts of urine contained substantial amounts of a material that did not move from the origin. Lung contained high concentrations of

drug-equivalents (175 µg/g at 24 hours), most of which (70-90%) was parent compound. Stomach, duodenum, jejunum, ileum, and large intestine contained similar concentrations of drug-equivalents, 50% of which were present as parent compound. In the cecum, parent compound represented 62% of the total drug-equivalents after 24 hours, but only 14-32% after 6 and 9 days, respectively.

Contents of the various tissues contained more drug-equivalents per gram than did the tissues per se. On day 3 after dosing, duodenum contained 18 µg of drug-equivalents/g while duodenal contents contained 40 µg of drug-equivalents/g. The same was true for jejunum (19 µg/g) and jejunum contents (57 µg/g), for ileum (19 µg/g) and ileum contents (129 µg/g), for cecum (24 µg/g) and cecum contents (251 µg/g), and for large intestine (10 µg/g) and large intestine contents (175 µg/g). In most cases the contents of tissues contained less parent drug than did the tissues (on the basis of percentage). The composition of the drug-equivalents found in the contents of the gut changed during the course of passage. In particular, the percentage of material remaining at the origin was high in bile, but was reduced substantially in the intestinal contents. Microorganisms are the suspected cause for these changes since the changes were of greater magnitude in the contents of cecum and large intestine where microorganisms are most commonly found.

The current studies have indicated that WR-180,409-$^{14}C \cdot H_3PO_4$ is readily absorbed from the gut, is rapidly distributed into various body compartments, is extensively metabolized, and is relatively slowly excreted.

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